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Claims

What is claimed is:

1. A biosensor for determination of analyte concentration in a test sample comprising:

5 a mixture for electrochemical reaction with an analyte; said mixture including an enzyme,
a mediator, and
an oxidizable species as an internal reference.

10 2. A biosensor as recited in claim 1 wherein said internal reference is defined as the reduced form of a reversible redox couple that has an equal or higher redox potential than that of said mediator.

3. A biosensor as recited in claim 1 wherein said mediator comprises 3-phenylimino-3H-phenothiazine.

4. A biosensor as recited in claim 3 wherein said internal reference comprises ferrocyanide.

15 5. A biosensor as recited in claim 4 wherein said ferrocyanide defining said internal reference and said mediator are oxidized at a first voltage potential and only said mediator is oxidized at a second voltage potential; said second voltage potential being less than said first voltage potential.

20 6. A biosensor as recited in claim 5 wherein said first voltage potential is about 400 mV and said second voltage potential is about 100 mV.

7. A biosensor as recited in claim 1 wherein said mediator comprises ferricyanide.

25 8. A biosensor as recited in claim 7 wherein said internal reference comprises ferrocyanide.

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9. A biosensor as recited in claim 1 wherein said mediator comprises ruthenium hexaamine.

10. A biosensor as recited in claim 9 wherein said internal reference comprises ferrocyanide.

5 11. A biosensor as recited in claim 10 wherein said enzyme comprises glucose oxidase.

12. A method of use of a biosensor including a mixture of an enzyme, a mediator, and an oxidizable species as an internal reference, said method comprising the steps of:

10 applying a first voltage potential in a first period;
providing a set delay period;
applying a second voltage potential in a final period following said delay period; and wherein said first voltage potential and said second voltage potential are selectively provided for oxidizing only said mediator or both said
15 mediator and said internal reference.

13. A method as recited in claim 12 wherein the step of applying a first voltage potential in a first period includes the step of applying a selected high first voltage potential in the first period for oxidizing said mediator and said internal reference.

20 14. A method as recited in claim 12 wherein the step of applying a first voltage potential in a first period includes the step of applying a selected low first voltage potential in the first period for oxidizing only said mediator.

15 15. A method as recited in claim 12 wherein the step of applying a second voltage potential in a final period following said delay period includes the step of applying a selected second voltage potential for oxidizing said mediator and said internal reference.

30 16. A method as recited in claim 12 wherein the step of applying a second voltage potential in a final period following said delay period includes the step of applying a selected second voltage potential for oxidizing only said mediator.

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17. A method as recited in claim 12 wherein the steps of applying said first voltage potential and applying said second voltage potential includes the steps of applying a selected voltage potential in a range between 100 mV and 400 mV.

5 18. A method as recited in claim 12 wherein the steps of applying said first voltage potential and applying said second voltage potential includes the steps of applying a selected first voltage potential in the first period for oxidizing both said mediator and said internal reference; and applying a selected second voltage potential for oxidizing only said mediator.

10 19. A method as recited in claim 12 wherein the biosensor includes a mediator comprising one of 3-phenylimino-3H-phenothiazine and ruthenium hexaamine; and wherein the internal reference comprises ferrocyanide; and wherein the steps of applying said first voltage potential and applying said
15 second voltage potential includes the steps of applying a selected first and second voltage potential for oxidizing only said mediator.

 20. A method as recited in claim 12 wherein the steps of applying said first voltage potential and applying said second voltage potential includes the steps of applying a selected first and second voltage potential for
20 oxidizing both said mediator and said internal reference; wherein said internal reference effectively anchoring a calibration intercept within a narrow range and said internal reference effectively maintaining a calibration slope for the biosensor.